Fungal Genetics and Biology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Fungal Genetics and Biology

journal homepage: www.elsevier.com/locate/yfgbi

How nutritional status signalling coordinates metabolism and lignocellulolytic enzyme secretion

Neil Andrew Brown [a,*], Laure Nicolas Annick Ries [a], Gustavo Henrique Goldman [a,b,*]

[a] Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, São Paulo, Brazil
[b] Laboratório Nacional de Ciência e Tecnologia do Bioetanol (CTBE), Campinas, Brazil

ARTICLE INFO

Article history:
Received 14 February 2014
Revised 26 June 2014
Accepted 28 June 2014
Available online xxxx

Keywords:
Carbon catabolite repression
Lignocellulolytic enzymes
Carbon starvation
Protein secretion
Saccharomyces cerevisiae
Ascomycete fungi

ABSTRACT

The utilisation of lignocellulosic plant biomass as an abundant, renewable feedstock for green chemistries and biofuel production is inhibited by its recalcitrant nature. In the environment, lignocellulolytic fungi are naturally capable of breaking down plant biomass into utilisable saccharides. Nonetheless, within the industrial context, inefficiencies in the production of lignocellulolytic enzymes impede the implementation of green technologies. One of the primary causes of such inefficiencies is the tight transcriptional control of lignocellulolytic enzymes via carbon catabolite repression. Fungi coordinate metabolism, protein biosynthesis and secretion with cellular energetic status through the detection of intra- and extra-cellular nutritional signals. An enhanced understanding of the signals and signalling pathways involved in regulating the transcription, translation and secretion of lignocellulolytic enzymes is therefore of great biotechnological interest. This comparative review describes how nutrient sensing pathways regulate carbon catabolite repression, metabolism and the utilisation of alternative carbon sources in *Saccharomyces cerevisiae* and ascomycete fungi.

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1. Introduction

Lignocellulose is the most abundant natural material on Earth and is seen as an attractive source of renewable energy. However, its recalcitrant nature impedes the efficient utilisation of this feedstock in green technologies. Lignocellulolytic fungi that colonise live and/or dead plant matter secrete an array of lignocellulolytic enzymes that efficiently degrade plant biomass, facilitating colonisation and providing a source of carbon to sustain growth. Therefore, such fungi must perceive this complex mixture of saccharides and adopt the most efficient strategy for coordinating polysaccharide degradation, carbon uptake and metabolism. Subsequently, several ascomycete fungi, including *Aspergillus* species and *Trichoderma reesei* have become the main commercial source of lignocellulolytic enzymes utilised in industry.

The ability to coordinate proliferation with cellular energetic status is a fundamental requisite for life and is conserved across all kingdoms. The catabolism of carbohydrates, through glycolysis, respiration or fermentation is the predominant route for energy production. Glucose catabolism represents the greatest energetic gain and is therefore the preferred carbon source for the majority of microbes. Subsequently, an ancestral capability to sense intra- or extra-cellular energy sources enables the coordination of cellular metabolism and the preferential consumption of glucose prior to other carbon sources, referred to as carbon catabolite repression (CCR), which is a common strategy utilised by microbes including budding yeasts and filamentous fungi.

The efficient production of biofuels and green chemistries from low cost plant residues, composed of varying saccharides, are all impaired via CCR. In the natural context the sole use of a single carbon source until exhaustion is beneficial. However, in the industrial situation, the autoinhibition of protein overproduction is undesirable. For example, the inhibition of lignocellulolytic enzyme secretion via the presence of readily consumable sugars, such as those released from lignocellulose deconstruction, impedes enzyme production and represents a major factor preventing the implementation of next-generation biofuel production (Himmel and Bayer, 2009). The efficient depolymerisation of lignocellulose into simple sugars represents the initial step for the majority of green chemistries. Therefore, how microbes sense the presence of differing carbon sources thus regulating CCR and lignocellulolytic enzyme production is the focus of this review.

* Corresponding authors. Address: Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Av. do Café S/N, CEP 14040-903, Ribeirão Preto, São Paulo, Brazil. Fax: +55 16 36024178.
E-mail addresses: neilbrown10@hotmail.co.uk (N.A. Brown), ggoldman@usp.br (G.H. Goldman).

http://dx.doi.org/10.1016/j.fgb.2014.06.012
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Please cite this article in press as: Brown, N.A., et al. How nutritional status signalling coordinates metabolism and lignocellulolytic enzyme secretion. Fungal Genet. Biol. (2014), http://dx.doi.org/10.1016/j.fgb.2014.06.012

The fundamental knowledge of nutrient sensing in microbes is predominantly founded upon studies of *Saccharomyces cerevisiae*, where the components and function of the relevant nutrient sensing signalling cascades that modulate growth and metabolism are known (for reviews see Rolland et al., 2001; Santangelo, 2006; Zaman et al., 2008). The equivalent genes in several model filamentous fungal systems have been identified but their regulation, mechanism and function are less well understood. The genes encoding for proteins involved in such nutrient sensing pathways have demonstrated a high level of conservation in *S. cerevisiae* and filamentous fungi, yet deviations in pathway composition and organisation do exist. The array and activity of the lignocellulolytic enzymes secreted by a filamentous fungal species depends upon the niche naturally occupied by a particular species. Saprophytic ascomycete fungal species including *Aspergillus nidulans*, *Neurospora crassa* and *T. reesei* have been widely adopted as the model organisms for the study of lignocellulolytic enzyme production (for reviews see Culleton et al., 2013; Glass et al., 2013; Kubicek et al., 2009). However, due to their efficient secretion systems, commercial enzyme cocktails are predominantly based on *A. niger* and *T. reesei* (Berka et al., 1991; Durand et al., 1988). Comparatively, the genome of *T. reesei* encodes fewer lignocellulolytic enzymes than other lignocellulolytic fungi (Martinez et al., 2008), while *A. niger* possesses a more versatile range of cellulases, hemicellulases and esterases that will become more important once undesirable lignocellulose pretreatment steps are abolished (Pel et al., 2007). Subsequently, *S. cerevisiae* has been used as a framework for the basis of this comparative review with the three model ascomycetes, *A. nidulans*, *N. crassa* and *T. reesei*, which focuses upon the three phases of lignocellulolytic enzyme regulation; (1) repression in the presence of preferred carbon sources, (2) derepression under carbon limitation and (3) induction of lignocellulolytic enzyme transcription and secretion. Finally, the remaining questions and application of such knowledge in *S. cerevisiae* and filamentous fungi within the industrial situation are discussed.

2. Signalling for the repression of lignocellulolytic enzymes in the presence of readily metabolised carbon sources

2.1. The mechanism and regulation of repression

Through the action of functionally conserved repressor proteins, CCR in fungi prevents the utilisation of alternative carbon sources via inhibiting the transcription of secreted and intracellular metabolic enzymes. The transcription of alternative carbon usage genes in *S. cerevisiae* and filamentous fungi is tightly controlled by a Cys_2-His_2 type DNA-binding zinc finger repressor protein, named Mig1p in *S. cerevisiae* and Cre1, CRE1 or CreA in filamentous fungi (Fig. 1) (Zaman et al., 2008; Shroff et al., 1997; Strauss et al., 1995). The *S. cerevisiae* Mig1p and *A. nidulans* CreA proteins show 70% identity (e-value 4e-29) within the double zinc finger domain. These repressor proteins in *S. cerevisiae* and filamentous fungi bind to GC boxes within the promoter of the repressed gene. The Mig1p binding motif (5′-ATAAAATGCGGGGAA-3′) is flanked by AT-rich regions that are proposed to assist in protein-induced target site bending (Lundin et al., 1994). In *A. nidulans* and *T. reesei,* two closely spaced CreA/CRE1 consensus motifs 5′-SYGGRG-3′ represent double binding sites that are key to repression (Cubero and Scazzocchio, 1994; Dowzer and Kelly, 1991; Takashima et al., 1996). In filamentous fungi additional repressor proteins have been identified, such as ACE1 of *T. reesei*, which has been shown to bind to the promoter of cellulase genes. The ACE1 homologue in *A. nidulans* StzA is described as a stress response factor (e-value 3e-86, 40.8% identity, 56.0% similarity; www.aspgd.org).

The industrial *T. reesei* RutC30 strain is a well-documented cellulase hyperproducer that is known to have a defective CCR system caused by a *cre1* truncation, in addition to enhanced protein secretion and altered protein glycosylation (IImen et al., 1996; Peterson and Nevalainen, 2012; Seidl et al., 2008). Similarly to the *cre1* deficient *T. reesei* strain, the *ace1* gene deletion strain demonstrates higher cellulase induction (Aro et al., 2003). In *N. crassa* CRE-1 has been also characterized and its role in the regulation of cellulase/hemicellulose gene expression has been analysed (Sun and Glass, 2011; Ziv et al., 2008) In *A. nidulans*, a complete *creA* gene deletion results in severe morphological defects, while partial gene disruption can enhance enzyme production (Shroff et al., 1997). However, the simple removal of the repressor protein is not sufficient to create an efficient system for enzyme hyperproduction. Therefore, a thorough understanding of the mechanism of CCR is required to enable the further enhancement of enzyme production.

2.1.1. Repression in S. cerevisiae

The nuclear localisation of Mig1p in *S. cerevisiae* cells regulates repressor function, with Mig1p being imported into the nucleus within minutes of the addition of glucose to derepressed cells (de Vit et al., 1997) (Fig. 1A). An additional level of gene regulation, which also governs CCR, is mediated through changes in nuclear chromatin structure, accompanied by short- or long-term alterations in transcriptional activity, which can be triggered by intrinsic cellular programs and/or environmental factors (Brosch et al., 2008). Chromatin organisation is influenced by nucleosome positioning and histone acetylation or methylation which in turn regulates gene expression via obstructing transcription factor binding (Li et al., 2007). The Tup1p/Ssn6p corepressor complex in *S. cerevisiae*, can be recruited by a number of DNA-binding proteins, including Mig1p, promoting histone deacetylation and nucleosome positioning (Smith and Johnson, 2000). The Mig1p repressor recruits the Tup1p/Ssn6p complex to the promoters of alternative carbon usage genes, enhancing repression via the modulation of nucleosomes positioning (Treitel and Carlson, 1995) (Fig. 1A). Chromatin distribution in the nucleus is non-random and the nuclear periphery is a key site in the transcriptional regulation of glucose-repressed genes. The reverse recruitment model describes how genes are activated by coming into contact with distinctly localised transcription factories that are tethered to nuclear pores (Menon et al., 2005). The Sucrose non-fermenting 1 (Snf1) complex is perinuclear during Mig1p derepression in *S. cerevisiae*, while a target gene *SUC2* associates with the nuclear pore when derepressed, compared to being highly mobile and randomly distributed throughout the nucleus when repressed (Sarma et al., 2007). Consequently, recent studies have shown that Mig1p physically interacts with the nuclear pore complex, which mediates its repressing function through a mechanism that is independent of Mig1p nucleocytoplasmic shuttling (Sarma et al., 2011).

2.1.2. Repression in filamentous fungi

In filamentous fungi, as with *S. cerevisiae*, the nuclear localisation of CreA/CRE1/Cre1 is also dependent on carbon availability (Fig. 1B). However, due to differences in lifestyle and the ability to catabolise various carbon sources, repressor localisation is not solely regulated by glucose in filamentous fungi. In *A. nidulans*, *Fusarium oxysporum*, *N. crassa* and *Sclerotinia sclerotiorum*, the regulation of CreA/1-GFP nuclear localisation plays a role in CCR (Brown et al., 2013; Sun and Glass, 2011; Jonkers and Rep, 2009a; Vautard-Mey et al., 1999). However, this carbon source response is only visible if the fusion protein is under the control of the native promoter. Similar studies of CreA/1-GFP fusion proteins in *A. nidulans* and *N. crassa*, under the control of a constitutive promoter (Roy et al., 2008; Sun and Glass, 2011), demonstrated constitutive nuclear localisation, irrespective of the presence of glucose,

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